Gel Permeation and Ion-exchange Chromatography of Proteins and Peptides

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This volume represents the first of a series which analyses recent advances in high performance liquid chromatography (HPLC). This first book focuses on the application of HPLC techniques to the purification and analysis of biologically active molecules such as proteins and peptides. The availability of this book will be welcomed by the many protein biochemists who have already adopted HPLC and wish to expand its use, and may convince others to invest in what is becoming an increasingly essential piece of laboratory equipment.

The chapters are written as experimental papers and provide a collection of alternative methods for the purification of a variety of proteins and peptides. Protocols and suggestions are given in a form which would allow the application of these techniques to other proteins of interest. Useful comparisons of HPLC and conventional 'soft gel' chromatography are often given, which show the use of HPLC giving higher resolution and better reproducibility in much shorter times. This often results in increased yields and sometimes higher specific activities of the final enzymes.

The chapters cover the difficulties and techniques involved in using some of the more common columns available for enzyme purification. Several chapters deal with the use of HPLC gel filtration. The effects of ionic strength, flow rate, sample concentration and tandem linking of a series of columns on the resolution of peaks are investigated in some detail. There are also interesting chapters concerning the use of HPLC gel filtration in the determination of native and subunit molecular weights.

Ion exchange chromatography is also dealt with in similar detail. Variables such as pH, solvent buffer, counter ion, particle size, pore size, column length, flow rate, temperature, and changing gradient slopes are all investigated with respect to resolution, yield and reproducibility.

The collected results from the experiments described provide a sound basis for choosing optimum working conditions for utilizing HPLC in the separation of proteins in general.

The book is finished off well with a final chapter on the technical aspects of biochemical HPLC. There are difficulties with conventional HPLC components in dealing with the corrosive salts and detergents used in the separation of complex mixtures of biologically active molecules. In this chapter the components and layouts of current biochemical HPLC systems are described and analysed in detail showing how conventional HPLC has been developed into the modern biochemical HPLC.

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